Prevalence of Menopause-associated Symptoms Throughout the Menopausal Transition in Women Aged 40 to 70 Residing in Spain and its Relationship with Physical Activity – the *Mujeres de Hierro* Study

Study Protocol and Statistical Analysis Plan

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Protocol history and version control

The original study protocol was approved by the Ethics Committee of the Universidad Politécnica de Madrid on 26 March 2026, before recruitment began on 18 April 2026. The present consolidated English-language protocol and Statistical Analysis Plan (Version 1, dated 2 June 2026) were finalized during ongoing recruitment, before database lock and before any formal analyses of study outcomes were conducted. Any subsequent amendments will be documented by version number, date, rationale, and affected sections.

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1. BACKGROUND AND RATIONALE

The menopausal transition constitutes a key physiological period in women's lives, characterized by progressive endocrine changes that significantly affect physical, psychological, and social well-being. In Spain, as in most European countries, increasing life expectancy means that a growing proportion of women will spend 40% or more of their lives in the postmenopausal stage[1]. This makes perimenopause and postmenopause stages of high public health relevance, not only because of the physiological changes they produce, but also because of their association with symptoms that can substantially reduce quality of life and affect daily functioning, work productivity, emotional well-being, and social and affective relationships[2].

Symptomatology associated with the menopausal transition is broad and heterogeneous. It includes vasomotor symptoms (hot flushes, night sweats), sleep disturbances, mood changes (irritability, anxiety, low mood), somatic symptoms (fatigue, joint pain), and urogenital manifestations (vaginal dryness, sexual dysfunction, urinary complaints), among others [2,3]. The intensity and type of symptoms vary considerably between women and also change over time, particularly during the transition from perimenopause to early postmenopause and, later, to more advanced postmenopause. However, both clinical practice and population-based research indicate that this symptomatology is frequently under-recognized. Many women normalize their discomfort or feel they lack adequate information, with considerable variability in the assessment and follow-up of these symptoms depending on socioeducational level and other social determinants[3].

Although epidemiological evidence indicates that menopausal symptoms can begin before the final menstrual period[3], the way in which different symptom domains evolve throughout the reproductive ageing continuum is still not fully understood. Specifically, whether the overall symptom burden follows comparable or domain-specific trajectories across reproductive stages remains unclear. Large cohort studies have contributed valuable knowledge on women's health during midlife[4–6]; however, many have examined only selected symptoms or restricted phases of reproductive ageing. Consequently, the multidimensional burden of menopausal symptoms across the full reproductive ageing continuum remains poorly characterized in large, real-world populations. This knowledge gap is consistent with the recognized global lack of large-scale epidemiological datasets capable of comprehensively mapping midlife health transitions, thereby constraining the design of targeted public health strategies[7].

A further issue is the relationship between physical activity, lifestyle, and menopausal symptomatology. Exercise and physical activity are a low-cost, accessible, non-pharmacological intervention with widely documented benefits across multiple dimensions of health [8]. In the context of menopause, several studies provide solid evidence that specific types of exercise are associated with a favorable frequency and intensity of vasomotor symptoms, mood, sleep quality, body composition, and cardiometabolic risk, in addition to contributing to the prevention of bone and muscle loss associated with the hormonal changes of menopause[9,10]. Nevertheless, studies focused on the association of physical activity on menopausal symptomatology are often based on small samples, conducted under conditions with limited applicability to the real-life context in which these women live[10].

In Spain, no study has jointly evaluated exercise habits, physical activity and the different domains of menopausal symptomatology, let alone in a large sample such as the one proposed in this study. Generating specific data would allow a better understanding of which exercise and physical activity patterns are associated with lower symptom burden, and at which stages the association might be most relevant, contributing to more precise and personalized recommendations[8]. In this context, updated, Spain-specific epidemiological information is essential. Although international studies and some national work exist, such as "*Flamenco Project"*[11], in many cases the available data derive from small samples, cover narrow age ranges that are not stratified by women's reproductive stages, or focus on specific symptoms without incorporating an integrated view of the phenomenon.

The largest study investigating the relationship between physical activity and menopausal symptomatology included 6,569 Australian women[4], but only in the 40–48-year age range, and therefore does not represent the majority of women in perimenopause and, above all, menopause. Another of the largest studies, based on the NHANES cohort with a sample of 3,755 women[12], evaluated the relationship between physical activity and depressive symptoms only. The remaining studies that consider broader age ranges and a more complete assessment of menopausal symptomatology rarely reach 1,000 participants, and most do not exceed 400, making it difficult to obtain a representative sample for each age band and reproductive status. Moreover, among different exercise types (i.e. aerobic, resistance or a combination), resistance exercise is of particular interest because midlife and reproductive ageing coincide with changes in muscle strength and physical function[13,14], yet perceived losses of strength and stamina have rarely been examined alongside other symptom domains.

Much of the available evidence derives from clinical samples or specific cohorts that do not always reflect the reality of the Spanish population. Menopausal symptomatology and its interaction with women's lifestyle depend on the geographical and cultural context in which women are situated[8,15]. To date, this limitation makes it difficult to estimate symptom prevalence in women residing in Spain of the different symptom domains, their evolution according to age and reproductive stage, their relationship with exercise and physical activity, and the associated factors that may act as modulators of symptom burden in women residing in Spain. Without robust, updated data, the ability to design preventive interventions, provide evidence-based recommendations, and plan health policies with a gender- and life-course-informed approach is limited.

From a public health perspective, the relevance of the project is twofold. First, it allows quantification of the magnitude of the problem, by estimating prevalence and describing the distribution of symptoms among participating women residing in Spain. Second, it allows progress in the identification of modifiable factors; exercise habits, physical activity and lifestyle habits constitute particularly relevant areas because of their preventive and therapeutic potential. If specific types of exercise, levels of physical activity or healthy lifestyle patterns are shown to be associated with lower symptomatology or better well-being, this evidence can inform health education programs, community interventions, primary care recommendations, and workplace health promotion strategies. It may also help identify sensitive stages during which introducing such changes could yield greater benefit, contributing to reduced unnecessary medicalization while supporting comprehensive, habit-centered approaches, without excluding the need for specific clinical interventions in certain cases.

Finally, accessible tools and platforms are needed to identify these habits and women's circumstances in a simple and applicable way, so that they can be implemented in primary care programs and facilitate transfer to the general population. The use of widely validated questionnaires, such as the MENQOL[16], through a purpose-built web platform, enables efficient, standardized, and scalable data collection, facilitating the recruitment of a large and diverse sample across different Spanish regions. Questionnaire-based research reduces risk to participants, minimizes participant burden, and captures information directly reported by women, which is essential for subjective variables such as menopausal symptoms.

In summary, the rationale for this project responds to a knowledge gap in an area of high prevalence and impact on quality of life. It will provide updated, Spain-specific data and evaluate a modifiable factor of high public-health interest, exercise and physical activity. The results will improve understanding of the evolution of symptomatology across the menopausal transition and provide a scientific basis for designing more effective, accessible interventions and recommendations, adapted to the Spanish sociocultural context.

2. AIMS

Primary aim
To determine the prevalence and severity of menopause-related symptomatology using the MENQOL questionnaire in women residing in Spain aged 40 to 70 years across the reproductive stages of the STRAW+10 framework.

Secondary aims

- To investigate the independent and joint associations of movement behaviors, including structured exercise, varying domains and intensities of physical activity, and sedentary time, with menopause-related symptom burden.

- To evaluate the relationship between anthropometric characteristics (e.g., BMI, weight status categories) and symptom severity, and assess how these associations fluctuate across STRAW+10 reproductive stages.

- To evaluate the relationships between underlying clinical status, including cardiometabolic, endocrine, and mental health conditions, and the prevalence of specific menopause symptom clusters.

- To assess the associations of musculoskeletal health indicators, functional metrics, and physical activity habits with estimated osteoporotic fracture risk across different reproductive stages and menopause etiologies (natural vs. induced).

- To examine sociodemographic inequalities and social determinants of health associated with varying degrees of menopausal symptom burden.

- To characterize the multidimensional profiles of women based on their menopausal hormone therapy history and examine the residual symptom burden associated with different management strategies.

- To identify multidimensional symptom phenotypes and clustering patterns based on the co-occurrence of physical, psychological, and vasomotor symptoms.

- To identify multidimensional symptom profiles and factors associated with a high symptom burden by integrating lifestyle, anthropometric, clinical, and sociodemographic characteristics.

3. METHODS / DESIGN

3.1 Study design

This is a nationwide, community-recruited, web-based cross-sectional study, based on data collected through self-administered questionnaires. The study is conducted in women residing in Spain aged 40 to 70 years.

The study will be reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cross-sectional studies [17] and the Checklist for Reporting Results of Internet E-Surveys (CHERRIES)[18].

3.2 Study population and eligibility criteria

The target population comprises women residing in Spain aged 40 to 70 years, regardless of reproductive stage. Participants can be classified as premenopausal, early perimenopausal, late perimenopausal, early postmenopausal or late postmenopausal, based on self-reported information on menstrual status and gynecological history collected through the online questionnaire.

Inclusion Criteria:

- Female sex.
- Age 40 to 70 years.
- Residence in Spain.
- Ability to understand and complete the online questionnaire.

Exclusion Criteria:

- Failure to provide electronic informed consent.
- Inability to understand the study information or complete the online questionnaire in Spanish.

The eligibility criteria described are intentionally broad and apply to enrolment in the study. They are designed to maximize reach, sample diversity, and geographical and sociodemographic coverage given the large-scale, multi-channel recruitment strategy (See section 3.3). For the primary analysis, following data cleaning, additional exclusion criteria are applied to define the primary analytic sample, as specified in the Statistical Analysis Plan (Section 4.2). Participants excluded from the primary analysis for reasons other than repeat submission remain part of the overall study dataset and, where appropriate and sample size allows, may be examined in dedicated secondary analyses.

For participants with identified repeat submissions, only the most recent submission is retained as the participant record used for analysis.

3.3 Recruitment and data collection procedures

Participants are recruited using non-probability sampling. Recruitment channels initially include institutional mailing lists and communication platforms distributed by the Universidad Politécnica de Madrid and the Cuerpos Serranos S.L. community network, as well as the University of Granada and other collaborating public institutions, universities, and partner organizations. Dissemination is further expanded through digital campaigns across social media and community-network channels, along with national mass-media coverage via television, press and radio broadcasts. Recruitment is additionally supported by snowball peer-referral dissemination, whereby participants share the study link within their own personal networks. No financial or material incentives are offered for participation.

The electronic questionnaire is implemented in a purpose-built web application. Before public launch, the research and technical teams repeatedly tested all relevant conditional pathways, response-validation rules, scoring algorithms, database mappings, and final-submission procedures. A technically stable version was subsequently usability-tested by 50 women aged 40–70 years who were independent of the research team. Feedback resulted only in minor refinements to the presentation, instructions, and study-specific content; validated instruments and scoring procedures remained unchanged, and all test data were deleted before recruitment began.

The survey is open and accessible through a public link or QR code, without registration, login, or an invitation-specific password. Recruitment is nationwide and is not restricted to any single Spanish region, consistent with the online, multichannel dissemination strategy described above. Questionnaire items are presented in a fixed order over 17 sequential screens, 14 of which contain questions. Adaptive branching results in between 80 and 147 displayed items, depending on the participant's responses. Eligibility, consent, and minimal identification items are mandatory, whereas other questions may be left unanswered after a reminder highlighting missing responses. Participants may navigate backwards and modify previously entered responses before final submission. Responses are captured automatically by the application without manual transcription.

Before starting the questionnaire, participants must read the study information sheet and provide electronic informed consent; only after explicit acceptance can participants access the full questionnaire, which collects sociodemographic and habits data, menstrual history, FRAX tool for fracture risk[19], the IPAQ (long form)[20] for physical activity assessment, and the MENQOL[16] questionnaire for menopause-related symptomatology (see Section 3.5). This procedure is strictly observational and non-interventional. It does not involve clinical manipulation or invasive procedures and there is no foreseeable physical risk associated with study participation.

The study is conducted in accordance with the applicable data-protection and research-ethics regulations, specifically Regulation (EU) 2016/679 (GDPR), Spanish Organic Law 3/2018 on Personal Data Protection and Digital Rights Guarantee, and Law 14/2007 on Biomedical Research. Data, including sociodemographic information, health history, menopausal symptomatology, and physical activity level, are collected remotely through a web application and backend hosted and managed through Lovable Cloud, which uses

Supabase's open-source foundation to provide database, authentication, storage, and related backend services. Within this infrastructure, data in transit are protected through HTTPS using TLS 1.3, and data stored at rest are encrypted using AES-256. Lovable Cloud operates under SOC 2 Type II-audited controls and an ISO 27001:2022-certified information-security management system. Upon final questionnaire submission, the completed questionnaire is automatically assigned a unique alphanumeric participant code and stored in pseudonymized form. Identifying data collected for consent and contact purposes are stored separately from questionnaire-derived health and lifestyle data, with restricted access.

3.4. Study variables

- Reproductive stage according to the STRAW+10 criteria.

- Prevalence and severity of menopause-related symptoms, assessed with the MENQOL questionnaire.

- Sociodemographic and health variables including age and anthropometry.

- Physical-activity and sedentary metrics derived from the IPAQ long form, and exercise type.

- Estimated fracture risk (10-year probability of major osteoporotic fracture and of hip fracture), derived from the fracture-risk items collected in the questionnaire.

3.5. Measurement instruments and reproductive-stage classification

3.5.1. Reproductive-stage classification

Reproductive status is determined in accordance with the Stages of Reproductive Aging Workshop +10 (STRAW+10) criteria[21], based on self-reported menstrual bleeding patterns and time since the final menstrual period. Women are categorized into one of five reproductive stages. Premenopause is characterized by regular menstrual cycles with no persistent changes in cycle length. Early perimenopause is defined by persistent variations of ±7 days or more in the length of consecutive menstrual cycles. Late perimenopause is characterized by the occurrence of amenorrhea intervals of 60 days or more within the preceding 12 months. Spontaneous postmenopause is confirmed retrospectively following 12 consecutive months of amenorrhea; within this group, early postmenopause spans from 1 to 8 years after the final menstrual period, and late postmenopause spans more than 8 years after the final menstrual period.

Women with permanent, irreversible treatment-induced amenorrhea (e.g., bilateral oophorectomy) are classified within the corresponding postmenopausal stage, consistent with International Menopause Society guidance[22]; although surgical menopause carries distinct long-term cardiovascular and metabolic risk implications, its underlying symptomatic profile is considered broadly comparable to that of natural menopause for the purposes of symptom-burden classification[23]. By contrast, cases where the underlying cause of amenorrhea prevents definitive classification via questionnaire data alone are categorized as unclassifiable and excluded from the primary analyses. This applies to women whose reproductive stage determination requires hormonal testing, as well as those with amenorrhea of ambiguous or potentially reversible origin — including hysterectomy with ovarian conservation, hormonal therapy or contraceptive use, and

medical treatment-induced temporary amenorrhea. Women reporting clinically incongruent combinations of responses relative to their nominal reproductive stage (e.g., a premenopausal participant reporting no ±7-day cycle-length variation, yet a date of last menstrual bleeding more than 60 days prior; or participants over 55 years old classified as premenopausal) are likewise excluded from the main analyses.

3.5.2. Menopause-Specific Quality of Life Questionnaire (MENQOL).

Menopausal symptomatology is assessed using MENQOL[16],an internationally validated questionnaire for measuring menopause-specific quality of life. The MENQOL comprises 29 items categorized into four domains: vasomotor (3 items), psychosocial (7 items), physical (16 items), and sexual (3 items). For each item, participants first indicate the presence or absence of the symptom over the previous month. If a symptom is present, participants rate the associated bother on a 7-point Likert scale ranging from 0 ("not at all bothered") to 6 ("extremely bothered"). In accordance with standard MENQOL scoring protocols, symptoms not experienced are assigned a score of 1, while experienced symptoms are converted to a continuous scale ranging from 2 to 8. Domain-specific scores are calculated as the mean of their respective constituent items, with higher scores reflecting greater symptom severity and poorer health-related quality of life. The Spanish-language version is used, ensuring cultural and linguistic adequacy[24].

3.5.3. Sociodemographic, health-history, and lifestyle variables

In addition to the validated instruments described above, the questionnaire collects sociodemographic, health-history, and lifestyle information through single- or few-item self-report questions, without a dedicated validated scale. Sociodemographic variables are collected through closed-ended items covering region of residence, educational level, employment status, and marital status. Health-history and clinical variables are collected through closed-ended items covering relevant prior conditions, current treatments, use of menopausal hormone therapy, other relevant gynecological history, and the fracture-risk items. Tobacco and alcohol use are each collected through a single item classifying current use and, where relevant, approximate frequency or amount. Regular physical exercise habits are assessed by determining whether participants currently engage in exercise two or more times per week and their predominant modality. Responses classify participants into four mutually exclusive categories: no regular exercise, predominantly aerobic or cardiovascular exercise (e.g., running, swimming, cycling, or group classes), predominantly strength exercise (e.g., free weights, machines, resistance bands, Pilates, or bodyweight exercises), or a combination of both modalities. Additionally, for participants reporting regular exercise, data on the specific weekly frequency (number of sessions per week) for each modality are also collected.

3.5.4. International Physical Activity Questionnaire (IPAQ, long form)

Physical activity level is measured using the IPAQ long form, an instrument validated for the adult population [20]. The questionnaire collects information on occupational physical activity, transport-related activity, domestic activity, leisure-time physical activity, and sedentary behavior. Data are converted into MET-minutes/week following official IPAQ scoring guidelines, allowing participants to be classified into low, moderate, or high physical activity categories. The Spanish-language version[25] is used, ensuring cultural and linguistic adequacy.

3.5.5. Fracture risk assessment questionnaire

Fracture risk is characterized using items derived from the FRAX tool, a clinical instrument that combines risk factors to estimate fracture probability [19]. Data collection follows the body-mass-index version of the instrument; femoral neck bone mineral density is not measured in this cohort and is therefore not incorporated into the resulting estimates. The items collected through the online questionnaire correspond to the standard FRAX clinical risk factors, self-reported by participants, including weight and height (used to derive body mass index), previous fracture, parental history of hip fracture, current smoking, long-term glucocorticoid use, rheumatoid arthritis, other conditions strongly associated with secondary osteoporosis, and alcohol intake.

4. STATISTICAL ANALYSIS PLAN

4.1 Sample size estimation

Sample size was estimated for the primary comparison of symptom prevalence across five STRAW+10 reproductive-stage categories using a chi-square test of homogeneity (k=5, df=4) and Cohen's w. Because directly comparable stage-specific estimates from Spanish cohorts were unavailable, planning assumptions were derived from the Australian Women's Midlife Years (AMY) Study[4]. This study used the validated MENQOL questionnaire[26] and classified premenopause and early and late perimenopause using an approach closely aligned with that of the present study. The estimates used referred to moderately-to-severely bothersome symptoms rather than symptoms of any severity.

As the AMY Study subdivided postmenopause by age rather than by early and late postmenopause, its published categories were aligned with the five-stage framework for planning purposes. Postmenopausal women aged younger than 55 years were used as an approximation of early postmenopause, whereas the prevalence estimates for women aged 55–59 and 60–69 years were combined using their respective sample sizes to approximate late postmenopause. These age-based groups were used solely to derive sample-size assumptions and were not considered formally equivalent to STRAW+10[21] postmenopausal stages.

Two scenarios were examined assuming equal allocation across the five reproductive-stage categories. For hot flushes, the symptom showing the greatest between-stage variation, the prevalence estimates were 8.8%, 20.3%, 37.3%, 26.1%, and 12.1%, yielding w=0.251 and a required analyzable sample of 246 participants at a two-sided α=0.05 and 90% power. A more conservative scenario based on poor memory used prevalence estimates of 23.0%, 36.9%, 36.5%, 30.5%, and 17.6%, yielding w=0.167 and a required analyzable sample of 550 participants. Allowing for up to 25% of recruited questionnaires being incomplete, invalid, or otherwise unavailable for analysis increased the corresponding recruitment requirements to 328 and 734 participants. Calculations were performed in R studio using the pwr package and pwr.chisq.test.

Because these calculations addressed only the primary comparison between reproductive stages, a substantially larger minimum operational recruitment target of 15,000 participants was initially estimated. This target was intended to increase the precision of stage-specific estimates, broaden geographical and sociodemographic coverage across Spain, and provide sufficient numbers for prespecified secondary, subgroup, and

sensitivity analyses. Recruitment beyond this minimum threshold was planned to further extend geographical reach, increase participation across key age, reproductive-stage, and sociodemographic strata, and improve the precision of estimates within clinically relevant subgroups. The 15,000-participant target represented a minimum operational threshold rather than a formal stopping rule or a fixed upper limit. Once this threshold will be reached, recruitment is planned to continue while the prespecified dissemination channels remained active and continued to yield eligible participants. Recruitment could be concluded once the planned dissemination campaign through universities, community networks, radio, press, television, and social media had been completed or those channels had been exhausted. Accordingly, the final sample size would be determined by participation throughout the planned recruitment campaign rather than by a predefined maximum. Recruitment continuation will not be contingent on interim analyses of symptom outcomes.

4.2 Data cleaning

Data cleaning will be performed prior to analysis, applying the following exclusion criteria sequentially in three stages (see also section 3.2). First, participants will be excluded if they submitted duplicate entries (only the most recent entry per participant retained), if the questionnaire was completed for less than 75% of items or if they do not reside in Spain. Second, reproductive stage will be classified according to the criteria described below (see section 4.3). Although data will be collected from all participants regardless of classification, women whose reproductive stage cannot be reliably determined from questionnaire responses alone will be excluded from the main analyses. This will first include women who report amenorrhea attributable to current pregnancy or breastfeeding, because such amenorrhea does not reflect the menopausal transition, followed by women with ambiguous circumstances that would require hormonal testing to resolve, such as amenorrhea following hysterectomy with ovarian conservation or amenorrhea attributable to hormonal treatments or contraceptives. Women who can be nominally classified according to the STRAW+10 criteria but whose responses to other questionnaire items are internally inconsistent with that classification will also be excluded. Finally, women currently using menopausal hormone therapy will be excluded from the main analyses to avoid confounding of the natural symptom profile by hormonal treatment aimed at alleviating those menopausal symptoms.

Technical safeguards will prevent repeated submission attempts from creating duplicate database rows. No persistent participant cookies, IP-address tracking, or log-file analyses will be used; instead, repeat submissions will be identified via normalized email addresses. The production database will exclusively store completed submissions, automatically discarding abandoned questionnaires.

4.3 Reproductive stage classification

Reproductive stage will be classified as previously described (see section 3.5.1) and analyzed as an ordered five-level categorical variable: premenopause, early perimenopause, late perimenopause, early postmenopause, and late postmenopause. The primary contrasts will be sequential, comparing each reproductive stage with the immediately preceding stage to characterize stage-related increases or decreases in symptom prevalence and severity. Additional analyses may include alternative reference

categories, selected pairwise comparisons, stage-specific analyses, or comparisons within clinically relevant subgroups, as appropriate.

4.4 Statistical analysis

Descriptive statistics will characterize the study sample. Continuous variables will be summarized as mean±SD or median (IQR) according to their distribution, and categorical variables as frequencies and percentages.

The primary outcome of the study is the prevalence and severity of menopause-related symptoms, measured with the MENQOL questionnaire, and its differences according to the five reproductive stages identified. Symptom prevalence will be estimated for the 29 MENQOL items, with corresponding 95% CIs, and compared across reproductive stages using regression models appropriate for binary or proportion outcomes (eg, generalized linear models with a binomial or Poisson distribution), allowing prevalence ratios or equivalent estimates to be obtained directly. Symptom severity will be analyzed as a continuous outcome using linear regression models. All primary models will be adjusted for age and BMI as a priori confounders. Estimated marginal means will be used to derive adjusted prevalence and severity estimates by reproductive stage.

Secondary analyses will examine the association of additional variables with symptom prevalence and severity, using the same general modelling approach as the primary analysis, with reproductive stage retained as an adjustment covariate where relevant. These will include: the association of exercise modality (e.g., no exercise, aerobic, strength, or combined) with symptom prevalence and severity; the association of physical activity level, characterized both continuously (MET-minutes/week) and by IPAQ category[20]; estimated fracture risk, including its potential interaction with reproductive stage and other variables of interest; and the association of sociodemographic variables (e.g., education, marital status, employment, geographical area) with symptom burden. Where sample size allows, secondary analyses may also be conducted in population subsets excluded from the main analyses, including women using menopausal hormone therapy and women who could not be classified by reproductive stage, to further characterize symptom burden in these specific groups.

Robust (heteroscedasticity-consistent) standard errors[27] will be used throughout the primary and secondary regression models to account for potential departures from model assumptions, and variance inflation factors will be examined to rule out problematic multicollinearity among covariates. Given the number of symptom-level and domain-level comparisons involved, appropriate corrections for multiple testing (e.g., false discovery rate procedures[28]) will be applied to reduce the likelihood of type I error, in addition to standard pairwise comparison adjustments where applicable.

When relevant, sensitivity analyses may be conducted to test the robustness of the primary findings, including unadjusted models, fully adjusted multivariable models incorporating additional relevant covariates, and, where sample size allows, analyses restricted to or stratified by clinically relevant subgroups. Complete-case analysis will be used for models incorporating the full covariate set. Owing to the non-probability sampling design, no survey, post-stratification, or calibration weighting will be applied. Estimates will therefore pertain strictly to the observed analytic sample and will not be interpreted as nationally representative population estimates; comparisons with external Spanish population benchmarks will be descriptive only.

All statistical tests will be two-sided, with statistical significance set at $p<0·05$, and estimates will be reported alongside 95% CIs. Given the cross-sectional design, findings will be interpreted as associations rather than causal relationships. Analyses will be performed using R software (R Foundation for Statistical Computing, Vienna, Austria) or equivalent statistical software.

5. RISKS AND BENEFITS

This study is observational and does not involve clinical intervention or invasive procedures; therefore, there is no foreseeable physical risk associated with study participation. Potential risks are limited to possible emotional discomfort arising from answering questions related to menopausal symptoms or health history, and to a minimal risk associated with the processing of personal and health data. Emotional discomfort is minimized by ensuring that survey questions regarding symptoms and personal history are not strictly mandatory, unlike the initial screening and consent items required for participation. Although participants are notified when a non-mandatory item is left unanswered, they retain the option to skip questions that cause discomfort. Data protection risks are adequately mitigated through strict compliance with Regulation (EU) 2016/679 (GDPR) and Spanish Organic Law 3/2018, the application of technical and organizational security measures, storage in secure technological environments, and access restricted exclusively to the authorized research team. Participants may withdraw from the study at any time without needing to justify their decision and without any resulting prejudice.

No direct clinical benefits are anticipated; however, as part of the study, participants receive an individualized report on their level of menopausal symptomatology, physical activity and estimated fracture risk, based on validated instruments and supported by scientific evidence, which may constitute an indirect benefit by promoting greater self-knowledge and understanding of their own health status. The individualized report is provided for informational purposes only, does not constitute a clinical diagnosis, and is not intended to replace consultation with a healthcare professional. In addition, the study presents relevant potential benefits at the collective and scientific level, by generating updated evidence on the prevalence and severity of menopausal symptomatology among participating women residing in Spain, analyzing its relationship with exercise and physical activity levels, and contributing to the design of future health-promotion strategies and lifestyle-based intervention programs. The project will also advance scientific knowledge in the field of women's health and the menopausal transition. Overall, given its non-interventional nature, the use of validated questionnaires, and the data-protection safeguards implemented, the risk–benefit balance is considered clearly favorable.

6. ETHICAL ISSUES AND DISSEMINATION

6.1 Institutional Ethics Committee approval

This study conforms to the ethical principles for medical research involving human subjects set out in the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Universidad Politécnica de Madrid (UPM) [approval number:

MDHPDSAALMALLDLTMEMD4A7AREEYSRCLAF-ABPL-20260311; date: 26 March 2026]. Recruitment began on 18 April 2026. The consolidated English-language study protocol and Statistical Analysis Plan were finalized on 2 June 2026, during recruitment but before database lock and formal outcome analysis, as detailed in the protocol history above.

6.2 Informed consent

All participants receive accurate information on the study and its data-collection procedures, and electronic informed consent is obtained from each participant before any questionnaire responses or study data were collected. Before starting the questionnaire, participants must read the participant information sheet and expressly accept the informed consent statements online; only after this explicit acceptance can they access the questionnaire. The information sheet clearly states that participants have the right to withdraw from the study at any time, without prejudice or need for explanation. Women unable or unwilling to give informed consent are not eligible for enrolment in this study.

Upon final questionnaire submission, the system automatically generates a unique alphanumeric participant code, which is provided to the participant and may subsequently be used to request the deletion of her submitted project data. The participant information sheet also provides the contact channels for the principal investigators, allowing participants to exercise their data-protection rights (access, rectification, erasure, restriction, portability, and objection) at any time after taking part in the study.

6.3 Confidentiality, data protection, and privacy

The confidentiality and privacy of all participants are cautiously respected and ensured throughout the conduct of the study. Submitted questionnaire data are pseudonymized upon final submission, when the system automatically generates and assigns a unique alphanumeric participant code. Identifying data collected for consent or contact management are stored separately from questionnaire-derived health and lifestyle data, with access restricted to the principal investigators. Only designated principal investigators retain access to the separately stored re-identification key. Researchers conducting data management and statistical analyses receive only the pseudonymized analytical dataset and do not have access to directly identifying information. All other data handling, processing, and statistical analysis is carried out using only the pseudonymized dataset, so that no participant can be identified by the research team at any stage of data analysis. Given that the study processes special categories of data under Article 9 of the GDPR (health data), reinforced security measures are applied from the design stage of the system (privacy by design and privacy by default), including encrypted HTTPS/TLS communications and secure, SOC 2 Type II–audited server infrastructure. Multiple application-, database-, and infrastructure-level safeguards were implemented to reduce spam, automated submissions, and technically invalid or inconsistent responses. These measures included payload and field validation, atomic data insertion, unique and traceable submission identifiers, restricted database permissions, single-use submission tokens, and bot and volumetric-attack filtering. Potentially implausible clinical values were flagged for subsequent quality-control review. Identified repeat submissions were traceable during data cleaning and were excluded from the primary analytical dataset, with only the most recent submission retained for each participant.

The Universidad Politécnica de Madrid and Cuerpos Serranos S.L. act as joint controllers for data-processing purposes, under Article 26 of the GDPR, and have formalized a joint-controllership agreement establishing their respective obligations, including management of participants' rights (access, rectification, erasure, restriction, portability, and objection) and the security measures applicable to the processing. Personal data are retained only for as long as strictly necessary for the conduct of the project and the resulting scientific analysis; once the project is completed, data may continue to be retained in pseudonymized form exclusively for scientific and statistical purposes, in accordance with applicable regulations. Results will be published exclusively in aggregated form, such that no individual participant can be identified.

6.4 Dissemination

Results will be presented in peer-reviewed, scientific journals and at international conferences. We aim to publish a main paper with the primary outcome data. Since it is a large project with numerous secondary outcomes, other specific manuscripts on each topic will be published. Beyond academic channels, we are committed to knowledge transfer to society. Key findings will be actively shared with the general public through press releases, radio, television, and social media campaigns, ensuring the research reaches all interested stakeholders and the wider population.

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